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First Report of Tomato Wilt Caused by *Ralstonia solanacearum* Biovar 1 in Puerto Rico

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Fig. 1. Bacterial wilt symptoms in tomato inoculated with *Ralstonia solanacearum*, biovar 1.

In 2010 bacterial wilt was detected in tomato (*Solanum lycopersicum* Mill) at a commercial farm in Coamo. Wilting of the foliage was observed in approximately 3,000 plants grown in a steep field. Bacterial streaming from the stem and an immunodiagnostic assay (BID-Rs ImmunoStrip Test, Agdia Inc., Elkhart, IN) conducted in situ indicated that the disease was caused by *Ralstonia solanacearum*. Similar symptoms were observed on tomatoes from two different farms in Aguada and Lares, showing breakdown of vascular tissue, necrosis of the buds and wilt. *Ralstonia solanacearum* was consistently isolated from the three locations on tetrazolium chloride

medium (TZC) (4), after 48 h at 28°C. Gram negative bacterial colonies from isolates 1287 from Aguada and 1290 from Lares were white with pink centers and did not grow at 40°C. Conventional and high-fidelity PCR using primers 759/760 (1) amplified a DNA fragment of 280 bp corresponding to *R. solanacearum*. The sequencing results showed 100% homology with *Ralstonia solanacearum*. The sequences of isolates 1287-9 (Gen Bank Accession number JQ771188), 1287-6 (Gen Bank Accession number JQ771190) and 1290-2 (Gen Bank Accession number JQ771189) correspond to an upstream region genomic sequence of *Ralstonia solanacearum*. Isolates were identified as biovar 1 by the production of acid from trehalose and dextrose and reduction of nitrite from nitrate without gas production. They did not produce acid from maltose, cellobiose, dulcitol, sorbitol, and mannitol. To prove Koch's postulates, forty 'Beefsteak' tomato seedlings (Laurel Seeds Company, San Juan, Puerto Rico) were grown in a commercial substrate (PROMIX). Tomato plants were inoculated with isolates 1287-9 and 1290-2 with 1 ml of a suspension (10-8 CFU/ml of isolates 1287-9 and 1290-2) by injecting the base of the stem. A negative control with sterile water was included. Plants were kept in a screen house at 23-31°C during May and June of 2011. Seven days after inoculation tomato plants developed flaccid leaves and wilted (Fig. 1). Disease severity was rated using the scale 1 to 4: 1 = healthy; 2 = wilting of lower leaves, stem necrosis and brown necrotic lesions; 3 = yellowing and wilting of leaves, exudate, and stem constriction or strangulation; and 4 = dead plant and production of bacterial exudate (2). *Ralstonia solanacearum* was reisolated from the stems of the symptomatic plants. Identity of the reisolated bacteria was verified with ELISA and PCR from bacterial colonies and plant tissue. There is not a

documented report of this disease in Puerto Rico, but an isolate from tomato is cited in a research paper using different strains of *Ralstonia solanacearum* (3). This is the first report of *Ralstonia solanacearum* biovar 1 affecting tomato in Puerto Rico. Early identification is essential to prevent its spread to other solanaceous species.

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